Assessment of cardiovascular disease risk in depressed women of reproductive and menopausal age

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Abstract: It is well documented that depression increases the risk of cardiovascular disease (CVD). Women of age 55 and younger with depression are more likely to have CVD. The present study aims to investigate CVD risk in depressed women of reproductive age (RA) and menopausal age (MA). Adult women of RA and MA were divided in to two groups; healthy and depressed. Women were screened for depression (ICD-10 criteria) at outpatients department of local psychiatric hospital. Fasting serum cortisol, estradiol and lipid profile levels were determined. Data was analyzed using two-way ANOVA followed by Newman's Keuls q-test. Total cholesterol (TC), low-density lipoproteins (LDL) and triglycerides (TGs) were raised in MA women however high density lipoprotein (HDL) and estradiol were lower as compared to RA women. Depressed RA women showed increased TC, LDL and HDL but decreased estradiol as compared to healthy women of similar age group. MA depressed women showed increased TC and LDL but decreased HDL and estradiol as compared to healthy controls. We found that MA depressed women had low HDL and estradiol as compared to RA depressed women. Circulating cortisol levels were increased in both depressed RA and MA women compared to respective healthy controls. Low HDL/LDL ratio was found in both healthy and depressed MA women when compared with respective RA women. A significant negative correlation of estradiol and cortisol was found in depressed RA women. It is concluded that low HDL/LDL ratio and hypercortisolemia in both healthy and depressed MA women make them more vulnerable to CVD.

Keywords: Depression; cortisol; menopause; cholesterol; cardiovascular disease.

INTRODUCTION

Estrogen and progestin play pivotal role in the life of women, influencing serotonergic neurotransmission and mood change (Bethea *et al*., 2002). Low estrogen levels have been associated with depression in menopause. The pathophysiology of major depression also involves impairment in negative feedback control of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in an increase in stress hormone (cortisol) levels. Depression is termed as a life threatening disorder, characterized by hypercortisolemia. Women with depression exhibit a higher degree of HPA axis activation, and the extent of dysregulation of HPA axis is greatest during menopause, when women suffer estrogen depletion (Weizman *et al*., 2012).

The effect of different types of stress on cholesterol concentration is of increasing awareness and significance. Several researchers have found that concentration of cholesterol tend to be significantly greater during stress than other times (Wertlake *et al*., 1958). The metabolic syndrome (MS) is defined as an assemblage of risk factors for cardiovascular diseases, and menopause is associated with an increase in metabolic syndrome prevalence. Hormonal changes associated with menopause cause a major effect on metabolism of plasma lipids and lipoproteins. Investigators have shown altered lipid profile in menopausal women who are estrogen deficient (Swapnali *et al*., 2011). Variation in estrogen concentration is encountered in different stages linked to the reproductive life of women and decrease levels of estrogen are connected with mood variations involving depression in women (McEwen and Alves, 1999). Dyslipidemia is a significant source of cardio vascular disease (CVD), which is consecutively the general cause of men and women morbidity (Castelli, 1988). The incidence of cardio vascular disease increases after menopause, since as women age they are more and more exposed to high numbers of major CVD risk elements, including an inadequate lipid profile, mental stress and also bodyweight (Gupta *et al*., 2007). The present study is designed to evaluate CVD risk in reproductive age (RA) and menopausal age (MA) women suffering from depression.

MATERIAL AND METHODS

This study was carried out from 2012-2014 at Clinical Biochemistry and Psychopharmacology Research Unit, Department of Biochemistry, University of Karachi. The subjects (n=100) were categorized into reproductive age (RA) women (n=50) having regular menstruation aged between 18-40 years and menopausal age (MA) women

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(n=50) with amenorrhea over one year aged between 52-65 years. The subjects were subcategorized into two groups, healthy and depressed, who met ICD-10 Criteria for depression at Psychiatry ward (without any other serious pathology and were free from any type of medication) were randomly selected, after an informed consent and ethical clearance from the ethical committee of Jinnah Postgraduate Medical Centre, Karachi.

Random fasting venesection (overnight fast of 12 hours) was carried out between 9:00-9:30 am to draw 10cc. of blood. The blood samples were centrifuged at 3000 rmp for 15 minutes. Serum was obtained and stored at –20^{o}C until analysis. Serum total cholesterol (TC), high density lipoprotein (HDL) and triglycerides (TGs) concentrations were determined by Randox® kit. Cortisol was measured using an Accu Bind ELISA test kit catalog number: 3625-300 by Monobind Inc USA. Estradiol was measured using an enzyme immunoassay test kit catalog number: BC-111 by Bio Check. Low-density lipoproteins (LDL) were calculated using formula.

Data was analyzed by two way ANOVA followed by Newman-Keuls statistics. Correlations between continuous variables were calculated using Pearson correlation coefficients. The level for significance was taken as $p < 0.05$.

RESULTS

Table 1 shows alteration in lipid profile in healthy and depressed RA and MA women. Data analyzed by two-way ANOVA, shows effect of age was significant on TC, HDL-C, LDL and TGs F=53.65 ($p<0.01$), F=188.55($p<0.01$), F=75.96 ($p<0.01$) and F=80.44 ($p<0.01$) respectively. Effect of disease was significant on TC F=36.65 ($p<0.01$), HDL F=50.15($p<0.01$) and LDL F=74.15 ($p<0.01$). There was no effect of disease on HDL and TGs levels. The interaction between the two (age x disease) was significant on HDL-C F=24.06 ($p<0.01$) and LDL-C F=6.49 ($p<0.05$).

Table 1: Serum lipid profile in healthy and depressed women of reproductive and menopausal age

Parameters	Healthy		Depressed		Two-Way ANOVA (df, 1, 96)		
	Reproductive Age	Menopausal Age	Reproductive Age	Menopausal Age	Age	Disease	Age × Disease
Cholesterol (mg/dl)	165.84±4.48	93.64±6.14**	187.04±2.99†	234.96±6.15**††	53.65 p<0.01	36.65 p<0.01	3.62 N.S
HDL (mg/dl)	53.12± 1.04	43.44±1.86**	58.5± 0.44††	38.08±0.31** ††	188.55 p<0.01	0.0005 NS	24.06 p<0.01
LDL (mg/dl)	75.8±1.78	101.28±4.72**	100.84±4.06††	148.24±5.24**††	75.96 p<0.01	74.15 p<0.01	6.49 p<0.01
TGs (mg/dl)	85.04±4.07	131.48±5.92**	94.0±5.43	139.76±4.97**	80.44 p<0.01	2.81 N.S	0.004 N.S

Experimental details are given in materials and method section. All values are means ± SEM n=25 in each group. The significance of the differences is indicated by, *p<0.05, **p<0.01 when menopausal age women were compared from respective controls, †p<0.05, ††p<0.01 when depressed women were compared from similar age healthy controls by Newman-Keuls Q statistics following two-way ANOVA. Abbreviations: HDL-C=high density lipoprotein Cholesterol; LDL-C=low-density lipoproteins Cholesterol and TGs=triglycerides N.S. = non-significant.

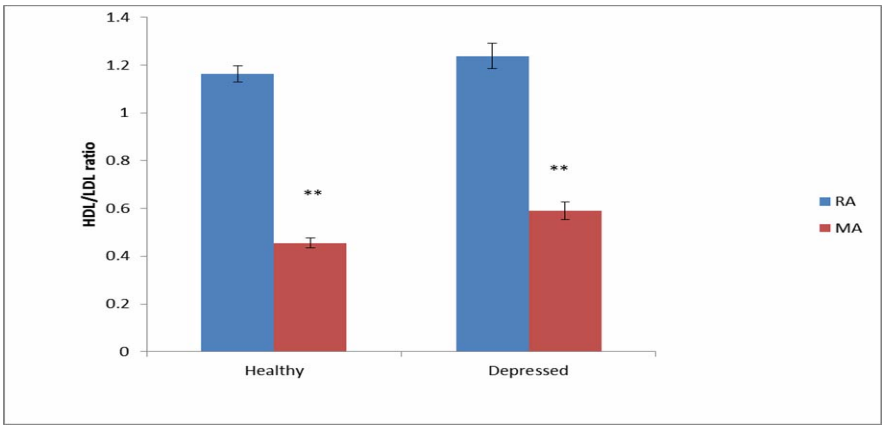


Fig. 1: Shows HDL/LDL ratio in healthy and depressed women of Reproductive age (RA) Menopausal age (MA) women. Values are means± S.E.M (n=25) the significance of differences is indicated by **p<0.01 when compared from respective control subjects, ††p<0.01when compared from similar age healthy controls by Newman-keuls Q statistics following two-way ANOVA.

Fig. 1 shows the ratio between healthy and depressed RA and MA women. Data analyzed by 2-way ANOVA indicates that the effect of age F=317.26 ($p<0.01$) and disease F=7.53 ($p<0.01$) was significant. But age x disease interaction was not significant.

Fig. 2 shows serum cortisol levels in normal healthy and depressed RA and MA women. Data analyzed by two way ANOVA followed by Newman-Keuls q-test. The results show that the effects of age were significant on cortisol F=174.53 ($p<0.01$). However the effect of disease F=999.93 ($p<0.01$) and age x disease interaction F=63.51 ($p<0.01$) was significant.

Fig. 3 shows serum estradiol concentrations in normal and depressed RA and MA women. Data analyzed by two way ANOVA shows that effect of age was significant on estradiol F=544.28 ($p<0.01$). Whereas the effect of

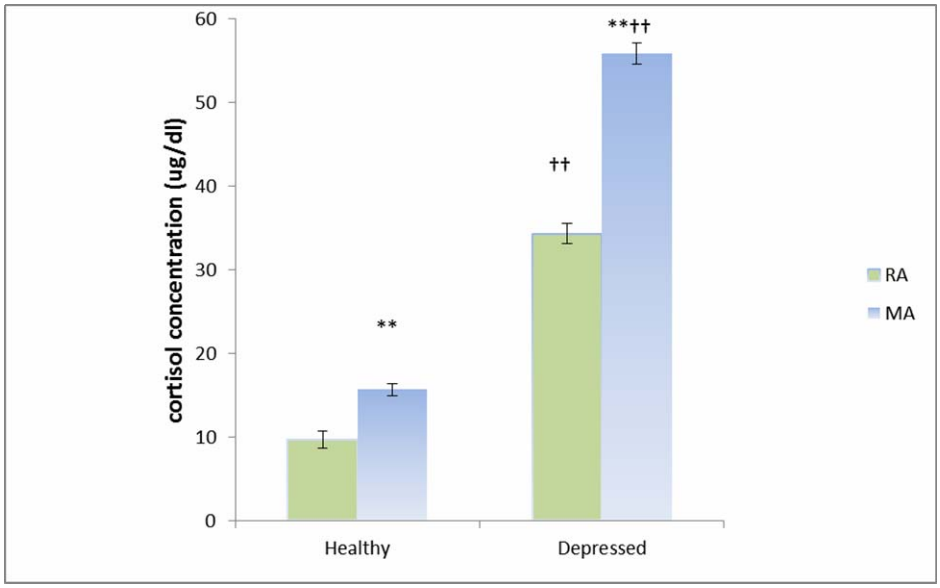


Fig. 2: Shows serum cortisol concentrations in in healthy and depressed women of Reproductive age (RA) Menopausal age (MA). Values are means± S.E.M (n=25) the significance of differences is indicated by **p<0.01 when compared from respective control, ††p<0.01 when compared from similar age healthy controls by Newman-keuls Q statistics following two-way ANOVA.

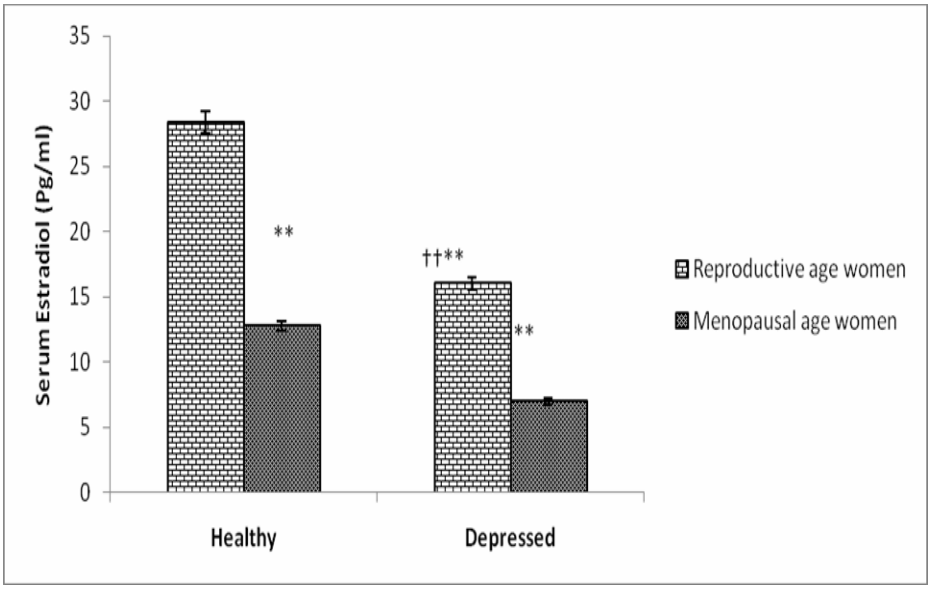
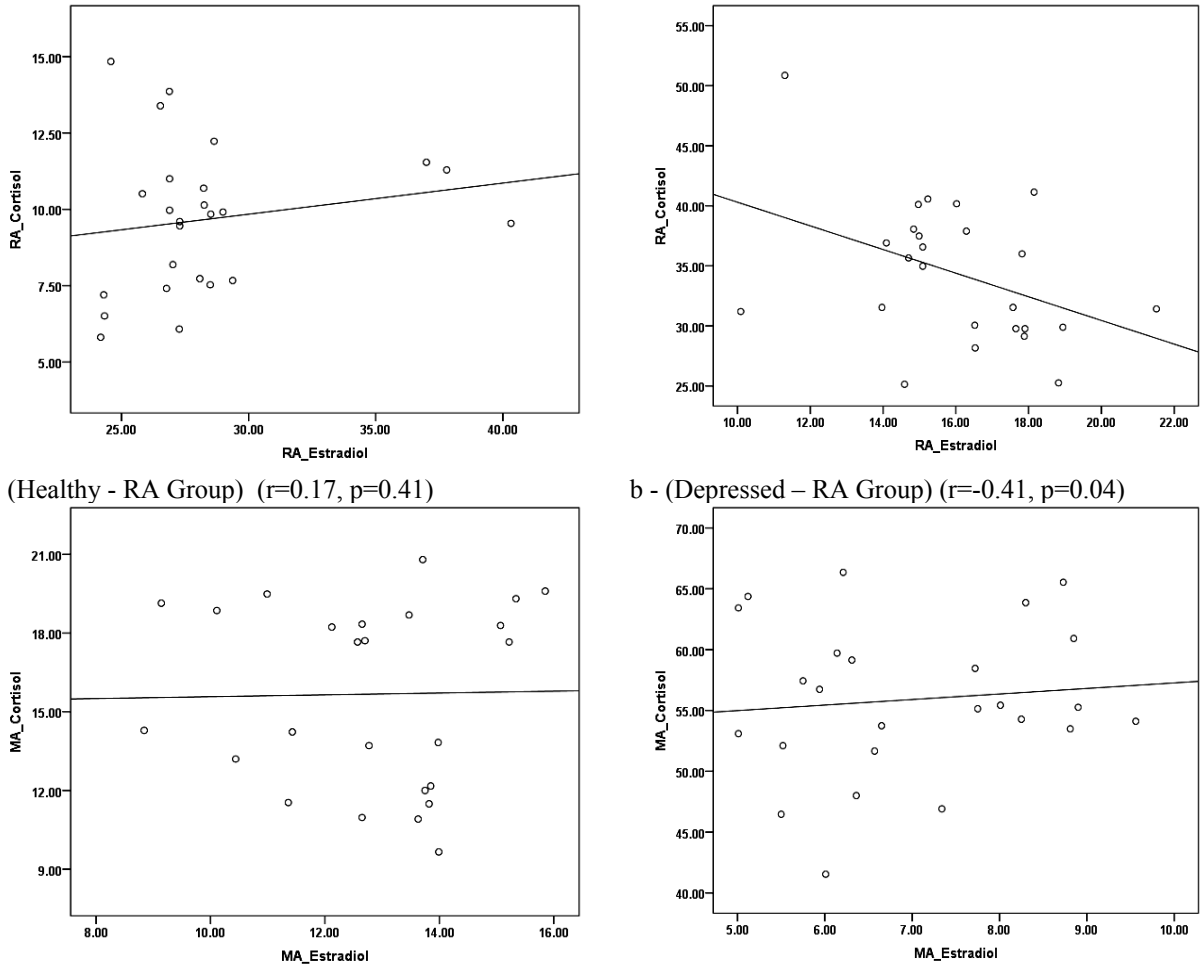


Fig. 3: Shows serum estradiol concentrations in healthy and depressed women of Reproductive age (RA) Menopausal age (MA). Values are means± S.E.M (n=25) the significance of differences is indicated by **p<0.01 when compared from respective control subjects, ††p<0.01 when compared from similar age healthy controls by Newman-keuls Q statistics following two-way ANOVA.



a- (Healthy - RA Group) (r=0.17, p=0.41)

b - (Depressed – RA Group) (r=-0.41, p=0.04)

c - (Healthy - MA Group) (r=0.02, p=0.93)

d- (Depressed - MA Group) (r=0.10, p=0.63)

Fig. 4 (a, b, c, d): Shows correlation of cortisol and estradiol in healthy and depressed RA and MA women. Correlations between continuous variables were calculated using Pearson correlation coefficients. The level for significance was taken as $p \leq 0.05$.

disease F=295.55 ($p<0.01$) and interaction between age x disease F=38.53 ($p<0.01$) was also significant.

Fig. 4 (a, b, c, d) shows correlation of estradiol and cortisol in healthy and depressed RA and MA women. Results indicate significant negative correlation of estradiol and cortisol (r=0.41, p=0.04) only in depressed RA in contrast correlations of estradiol and cortisol in healthy and depressed MA and healthy RA was not significant.

DISCUSSION

The prevalence of the metabolic syndrome (MS) increases with menopause and may partially explain the apparent acceleration in CVD after menopause. Altered lipid profile makes MA women susceptible to atherosclerosis, the major cause of death for nearly 53% of all deaths in women over 50 years of age (Shilpa *et al*., 2001). The present study (table 1) shows higher TC, LDL, triglycerides levels with low HDL and estradiol levels (fig. 3) in healthy MA women when compared with healthy RW controls, results are in accordance with findings as reported earlier (Ushiroyama *et al*., 1993). Increased body mass of MA women is because of greater fat deposition with elevated release of free fatty acids into the blood circulation, providing substrate for hepatic triglyceride in addition to triglyceride rich lipoprotein production (Tanko *et al*., 2005). Menopause causes decreases of HDL concentrations. Deficiency of estrogen in MA women may have greatest activity of post heparin hepatic lipase and promotes the uptake of HDL and also enhances the catabolism of HDL, reducing plasma HDL levels (Arora *et al*., 2006). According to Arca *et al*., (1994) decrease in estrogen secretion with the cessation of ovarian function contributes to higher LDL level in MA women. Estrogen increases hepatic synthesis of LDL receptor for Apo-β resulting in increased LDL-C uptake decreasing circulating LDL levels (Wild *et al*., 1995). Furthermore decreased gonadal activity decreases LDL

degradation in menopause. The present study also shows (table 1, fig. 3) that TC, LDL and TGs were significantly higher and estradiol levels were lower in depressed MA women. It is observed that during physical and psychological stress there are alterations in the levels of serum lipids to meet the extra metabolic demands of body tissues. During stress the stored cholesterol in adipose tissues is usually released into plasma and is the main source of the hypercholesterolemia. The acquisition of LDL and HDL cholesterol and an increase in cholesterol synthesis is pivotal for adaptation to stress. The relation between depressive symptoms and cholesterol is mainly present in older age groups. According to Placido *et al.* (2012) and Bittner (2002), MA women may be susceptible to depression due to decreased estrogen and its association with lipid profile. Present study shows a strong relationship between raised serum lipids and declined levels of estradiol in depression. RA women suffer from hypercholesterolemia that can be handled by them because the reproductive hormones estrogens serve as a natural protection against cardiovascular risk by raising HDL. Antidepressants have an effect on lipid milieu too facilitating the process of normalization and maintenance of homeostasis (Gity and Bano, 2013). The increase in serum TC and LDL-C in depressed MA women assume a great significance since this pattern is associated with CHD. On the other hand present study (fig. 2) shows that decreased (0.46, 0.59) HDL/LDL ratio in healthy and depressed MA women, suggestive of increased cardio vascular (CV) risk in MA women is a significant predictor for development of atherosclerosis (Kanwar *et al*., 2014). Consequently, in MA women low levels of estrogen are likely to decrease HDL/LDL ratio (Eapen *et al*., 2010).

Cortisol is released in response to adrenocorticotropic hormone ACTH from adrenal cortex. Over production is associated with impaired HPA axis negative feedback (Piwowarska *et al*., 2009). In present study we have found high level of cortisol in healthy MA women. These women are more prone to nocturnal increases in cortisol associated with mild stressors. Estrogen helps to regulate the normal morning cortisol peak and therefore helps stabilize night time sleep (Moe *et al*., 2001). Increased levels of cortisol have been linked with poor health with lower bone density in aging women, memory loss, sleep disturb and severe hot flushes. On the other hand, present findings are consistent with earlier report (fig. 1) that cortisol levels are high in depressed MA as compared to healthy MA women (Bhagwagar *et al*., 2005). Cortisol is an important factor inducing tryptophan 2, 3 dioxygenase, the first rate limiting enzyme of tryptophan oxidative metabolisms reducing available tryptophan for serotonin synthesis (Green and Curzon, 1968). Females are at higher risk of major depression, as a result of heightened sensitivity to intense hormonal fluctuations. Cortisol is synthesized from cholesterol as its essential precursor. It is equally possible that elevated cholesterol may cause an elevation in cortisol concentration. Our results indicate that MA women have high level of cortisol in depression as compared to depressed RA. Depressed women exhibit hypercortisolemia and a higher degree of HPA axis activation than depressed women and the extent of dysregulation of the HPA axis is greatest during the menopause (Shin *et al*., 2008). Interestingly, present result shows that increased level of cortisol in depressed RW than healthy controls is in agreement with that reported earlier (Bano *et al*., 2004). In post-menopausal women increased cortisol level is associated with known risk factors for cardiovascular disease, such as insulin resistance and decreased HDL-cholesterol level (Cagnacci *et al*., 2011).

As regard to correlation, present findings (fig. 4 a, b, c, d) show that significant negative correlation of estradiol and cortisol ($r=0.41$, $P=0.04$) in depressed RA. The reason could be due to estrogens stimulate the HPA axis. In addition, HPA axis responsiveness is greater in women that in men (Gallucci *et al*., 1993). Estrogen directly stimulate CRH gene promoter and the central nor adrenergic (norepinephrine system) which may helps to explain adult women slight hypercortisolism increases in effective anxiety, eating disorder, mood cycles and vulnerability to autoimmune and inflammatory disease. All of which follow estradiol concentrations fluctuation. Estradiol down regulates glucocorticoids receptor binding in the anterior pituitary, hypothalamus and hippocampus. This tends to increase HPA axis activity by interfering with glucocorticoids negative feedback whereas progesterone opposes these effects (Peiffer *et al*., 1991). Thus alterations in estradiol levels during normal menses perimenopause and menopause alter the regulatory feedback loop and adaptation overtime developed as a new equilibrium established in the relationship. Overtime these changes increase the incidence of mood alteration eating disorders, anxiety, depression weight changes and inflammatory and immune disease.

CONCLUSION

It is concluded that both RA and MA depressed women have elevated levels of cortisol. However both healthy and depressed MA women have high risk to develop CVD due to dyslipidemia and low HDL/LDL ratio. Menopause is associated with an increase in MS prevalence. It is recommended to screen the MS component especially in depressed MA women before prescribing the antidepressant therapy, considering the class of antidepressants that may cause the least modulations of the lipid milieu. In addition to choosing an appropriate antidepressant, the clinician should check the lipid profiles and related parameters these patients on a regular basis as MA depressed women are more vulnerable to cardiac mortality.

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